The effects of 30 days resveratrol supplementation on adipose tissue morphology and gene expression patterns in obese men.

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SHORT COMMUNICATION

The effects of 30 days resveratrol supplementation on adipose tissue morphology and gene expression patterns in obese men

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Polyphenolic compounds, such as resveratrol, have recently received widespread interest because of their ability to mimic effects of calorie restriction. The objective of the present study was to gain more insight into the effects of 30 days resveratrol supplementation on adipose tissue morphology and underlying processes. Eleven healthy obese men were supplemented with placebo and resveratrol for 30 days (150 mg per day), separated by a 4-week washout period in a double-blind randomized crossover design. A postprandial abdominal subcutaneous adipose tissue biopsy was collected to assess adipose tissue morphology and gene expression using microarray analysis. Resveratrol significantly decreased adipocyte size, with a shift toward a reduction in the proportion of large and very-large adipocytes and an increase in small adipocytes. Microarray analysis revealed downregulation of Wnt and Notch signaling pathways and upregulation of pathways involved in cell cycle regulation after resveratrol supplementation, suggesting enhanced adipogenesis. Furthermore, lysosomal/phagosomal pathway and transcription factor EB were upregulated reflecting an alternative pathway of lipid breakdown by autophagy. Further research is necessary to investigate whether resveratrol improves adipose tissue function.

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INTRODUCTION

Obesity is reaching epidemic proportions and is associated with insulin resistance and an increased risk for type 2 diabetes mellitus.[1] Polyphenolic compounds, such as resveratrol, are currently an area of intense investigation because of their ability to mimic effects of calorie restriction. Pronounced effects of resveratrol have been reported on lipolysis, adipogenesis and inflammation in isolated adipocytes and on adipose tissue of murine animals.[2] These effects may possibly reflect changes in adipose tissue function and an improved insulin sensitivity.[3] On the other hand, a mice study with resveratrol showed an inhibition of adipogenesis.[4] Although the latter finding has been implicated in the prevention of body fat accumulation in this study, this may also reflect impaired adipogenic potential resulting in a reduced adipose tissue expandability and insulin resistance.[5]

A recent human study observed no changes in adipose tissue mass (total, subcutaneous or visceral fat), adipose tissue or muscle metabolic and inflammatory gene expression profiles and insulin sensitivity after 4 weeks of a high daily dose of resveratrol (1500 mg per day) in obese healthy men.[6] In another study of Yoshino *et al.* in non-obese postmenopausal women, resveratrol supplementation (75 mg per day) did not affect liver, muscle or adipose tissue insulin sensitivity or its putative molecular targets in adipose tissue or muscle.[7] In contrast, in a recent study in our laboratory, it was shown that resveratrol supplementation for 4 weeks (150 mg per day) induced metabolic changes in liver and skeletal muscle metabolism in obese humans, mimicking the effects of calorie restriction.[8] These data may suggest that the resveratrol-induced metabolic effects may depend on the administered dose or to what extent the metabolic state is compromised.

The general aim of the present work was to gain more insight into the effects of 30 days resveratrol supplementation (150 mg per day) on adipose tissue morphology and the transcriptional profile using microarray analysis in healthy obese men.

PATIENTS AND METHODS

Study design

The current study was part of a previously published randomized double-blind crossover study.[8] Eleven obese, healthy men participated in two trials for 30 days: a placebo and resVida (150 mg per day trans-resveratrol (99.9%; DSM Nutritional Products, Kaiseraugst, Switzerland)) condition, with a 4-week washout period. At the end of both intervention periods, a subcutaneous adipose tissue biopsy was taken 6 h after ingestion of a high-fat liquid test meal.[8] The original study protocol was approved by the Medical Ethical Committee of Maastricht University Medical Center and written informed consent was obtained before study participation.

Abdominal subcutaneous adipocyte size

Processing and analysis of subcutaneous adipose tissue biopsies for adipocyte size have been described in detail previously.[9] Briefly, an adipose tissue needle biopsy (~1 g) was collected 6–8 cm lateral from the umbilicus. Part of the adipose tissue was fixed overnight in 4% paraformaldehyde and embedded in paraffin for histological sections

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(8 μm), whereas the other part was snap frozen in liquid nitrogen and stored at −80 °C. Sections were stained with hematoxylin and eosin. Digital images were captured and computerized morphometric analysis of individual adipocytes was performed in a blinded manner.

Adipose tissue gene expression

Microarray processing. Total RNA was extracted from frozen adipose tissue specimens using TRIzol reagent (Invitrogen, Breda, The Netherlands) and purified on columns using the Qiagen RNeasy Micro Kit (Qiagen, Venlo, The Netherlands). Total RNA (100 ng per sample) was labeled by Whole-Transcript Sense Target Assay and hybridized to human whole-genome Affymetrix Gene 1.1 ST arrays targeting 19 793 unique genes (Affymetrix, Santa Clara, CA, USA).

Microarray data analysis. Quality control and data analysis have been described in detail previously.[8] Individual genes were defined as changed when comparison of the normalized signal intensities showed a *P*-value <0.05 in a two-tailed paired intensity-based moderated t-statistics (PMID:17177995). These analyses were performed within MADMAX system (PMID:21778530). Further functional data analysis was performed on the filtered data set (>5 arrays with signal intensity >20) with Gene Set Enrichment Analysis (http://www.broad.mit.edu/gsea/) and gene sets were selected based upon a FDR *q*-value <0.25. A transcription factor analysis was performed on the differentially expressed genes (*P*-value <0.05) with Ingenuity Pathway Analysis (June 2012, Ingenuity Systems, Redwood City, CA, USA). Array data have been submitted to the Gene Expression Omnibus GSE42432.

Statistics

Student's paired *t*-test was used to compare placebo and resveratrol supplementation in normally distributed data. A *P*-value <0.05 was considered statistically significant. Data are reported as mean ± s.e.m. Statistical analyses were performed using the statistical program SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

Eleven obese male volunteers aged 40–65 years and body mass index between 28 and 36 kg m^{-2} participated in this study. Other subject characteristics and effects of 30 days resveratrol on plasma biochemistry have previously been reported.[8]

Resveratrol decreased abdominal subcutaneous adipocyte size

Mean adipocyte size was decreased after 30 days resveratrol (65.0 ± 4.4 μm) compared with placebo (74.7 ± 3.5 μm; Figure 1c). This was attributed by a shift toward a lower proportion of very large adipocytes (>90 μm) and an increased proportion of small adipocytes (<50 μm). The proportion of large (70–89 μm) adipocytes also tended to be lower after resveratrol supplementation (Figure 1d). A representative image is shown in Figures 1a and b.

Microarray analysis

From the 19 793 genes on the array, 12 676 genes were expressed in adipose tissue. Resveratrol supplementation resulted in changed expression of 582 genes, of which 290 were upregulated and 292 were downregulated.

Gene set enrichment analysis

Gene sets that were downregulated by resveratrol belonged to pathways involved in cell–cell junction, Wnt signaling, angiogenesis, G protein coupled receptors and Notch signaling (Figure 2a). Gene sets upregulated by resveratrol were associated with lysosome, phagosome, inflammation, glucose/hexose transport and cell cycle (Figure 2b). Upregulated pathways associated with inflammation included interferon signaling, antigen processing and presentation, T-cell receptor signaling and nuclear factor κB activation (Supplementary Table 1). Core-enriched genes per up- and down-regulated pathways are presented in Supplementary Table 2.

Transcription factor analysis

Several transcription factors involved in interferon signaling were identified as potentially activated (Supplementary Table 3). Furthermore, the nuclear factor κB complex involved in inflammation, PPARδ and transcription factor EB (TFEB), which links autophagy to lysosomal biogenesis, were shown to be activated.[10]

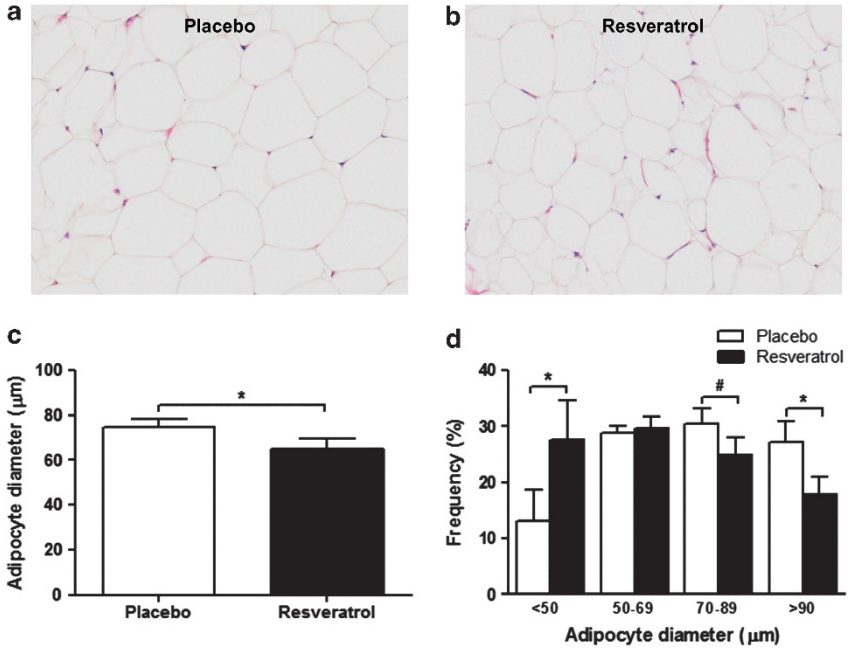

Figure 1. Adipocyte size measurement after placebo and resveratrol supplementation. Representative adipose tissue sections after placebo (**a**) and resveratrol (**b**) supplementation (×20 magnification). (**c**) Mean adipocyte diameter. (**d**) Adipocyte size distribution. $N=8$. Values are means ± s.e.m. $^{\#}P<0.1$, $^{*}P<0.05$.

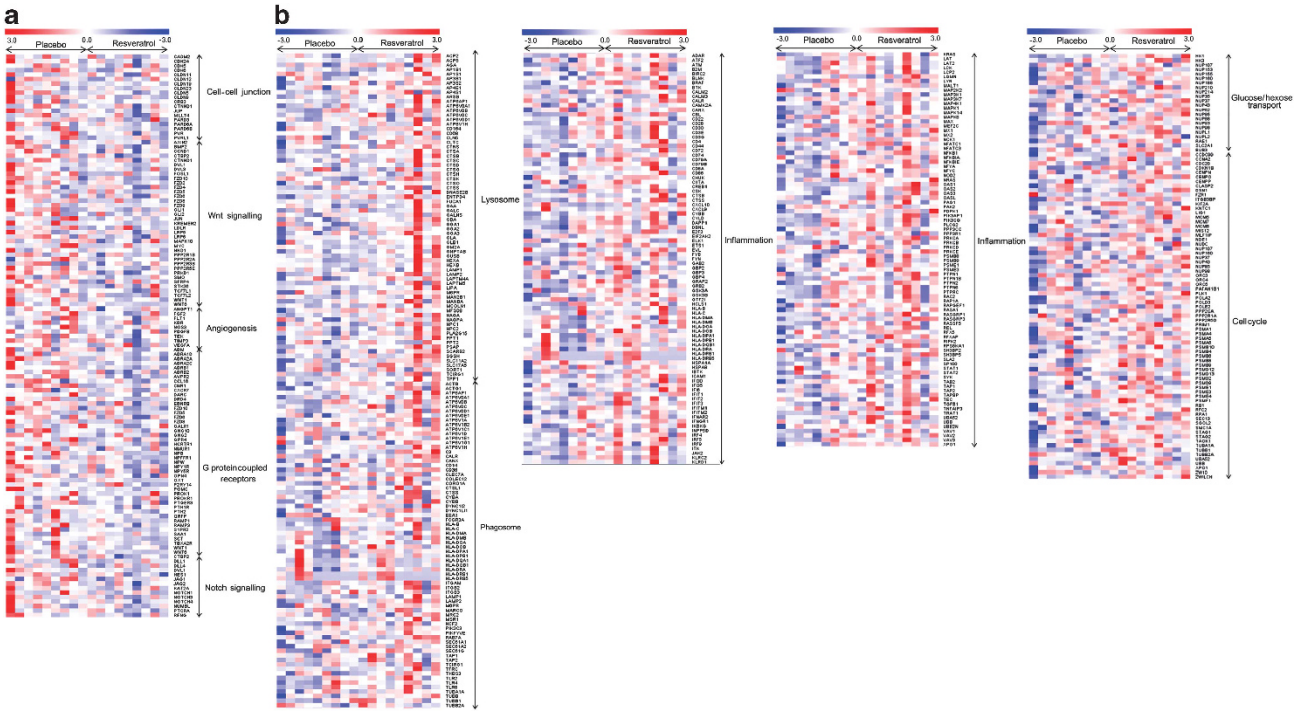

Figure 2. Up- and downregulated gene sets after resveratrol supplementation using gene set enrichment analysis. (**a**) Downregulated gene sets after resveratrol supplementation. (**b**) Upregulated gene sets after resveratrol supplementation. The *z*-score was calculated by subtracting the mean expression value for each transcript from each of the values and then dividing the resulting values by the standard deviation. Only the core-enriched genes are represented in this figure. Color in the heat-maps reflects the relative transcript abundance level with red being higher and blue lower than the mean transcript abundance value.

Transcription factors that were identified as inhibited included Notch 4 and SMAD4 (Supplementary Table 3). The transcription factor hypoxia-inducible factor 1-α was also inhibited.

DISCUSSION

Resveratrol significantly decreased adipocyte size, with a shift toward a lower proportion of large and very large adipocytes and an increased proportion of small adipocytes. Gene expression analysis indicated an increased adipogenesis and an altered pathway of lipid breakdown by autophagy. Together, these data suggest that the reduced mean adipocyte size may underlie the previously reported improved insulin sensitivity in these subjects, as a reduced adipocyte size in combination with an improved adipogenesis may be related to an improved insulin sensitivity in humans.[11]

Microarray analysis indicated that the Wnt signaling and Notch signaling pathway were downregulated after resveratrol supplementation. Inhibition of these pathways has shown to result in adipogenesis of preadipocytes and/or multipotent precursor cells,[12,13] although the exact role in adipogenesis needs to be further elucidated. Gene sets associated with cell cycle were upregulated by resveratrol. This indicates that an increased adipogenesis could possibly explain the reduced adipocyte size after resveratrol. In contrast to our *in vivo* observations, *in vitro* experiments performed in murine and human adipocyte cell lines have shown that resveratrol inhibited proliferation and adipogenic differentiation.[14,15] The discrepancy between *in vitro* results and the present data may be explained by differences in resveratrol concentrations, acute *in vitro* vs chronic *in vivo* administration and the presence of stromal vascular cell types (for example, inflammatory cells) in the *in vivo* situation.

Our data show that the angiogenesis pathway was downregulated in human adipose tissue after resveratrol supplementation. Furthermore, the transcription factor hypoxia-inducible factor 1-α whose activity is induced by hypoxia, insulin and nitric oxide[16] was also downregulated. These data are consistent with studies showing that resveratrol possesses anti-angiogenic effects,[17] but further research is necessary to determine the exact role of resveratrol on angiogenesis in adipose tissue.

Interestingly, activation of a lysosomal pathway of lipid breakdown was demonstrated. Until recently, mobilization of lipids by the classic lipolytic pathway has only been attributed to lipid droplet-associated proteins and lipases. Lately, an alternative pathway of lipid metabolism has been proposed through the lysosomal degradative pathway of autophagy. Major support for the involvement of autophagy is the activation of TFEB following resveratrol supplementation in the present study. TFEB controls multiple crucial steps of the autophagic pathway.[10] Studies in hepatocytes and liver have demonstrated lipid breakdown by autophagy.[18] In preadipocytes, inhibition of autophagy has shown to block adipogenesis.[19] Therefore, in the present study, the lysosome and phagosome pathways, may have contributed both to an induction of adipogenesis as well as to alternative lipid breakdown, possibly contributing to the shift toward an increased proportion of small adipocytes after resveratrol.

In the present study, several pathways involved in the immune response were upregulated after resveratrol treatment. This may reflect an increase in cellular stress induced by the reduction in adipocyte size caused by traction forces between the adipocyte and the surrounding extracellular matrix. This may induce upregulation of inflammatory genes and pro-inflammatory adipokine production, as shown after weight loss.[20] On the other hand, upregulation of immune response pathways may be a physiological response to a higher lipid turnover (that is,

lysosomal lipid breakdown). We previously observed in these subjects a decrease in plasma interleukin-6 and tumor necrosis factor-α concentrations and a downregulation of gene sets associated with immune response in skeletal muscle following resveratrol supplementation.[8] These findings indicate that the increased immune response in adipose tissue could represent a local rather than a systemic response.[8]

The present results show that resveratrol increased pathways in the adipose tissue involved in glucose and hexose transport as evidenced by GSEA. However, *in vitro* data concerning the effects of resveratrol on glucose transport are conflicting.[15] It needs to be further established if the observed increase in glucose/hexose transport pathway, translates into a functional increase in glucose uptake.

As indicated above, resveratrol clearly affected adipose tissue gene expression profiles. This is in contrast with two recent human trials, which did not show any effects on adipose tissue metabolism or gene expression after resveratrol supplementation.[6,7] The reason for this discrepancy may possibly lie in the administered dose, which was very high in the study of Poulsen *et al.* (1500 mg per day) as compared with our study or the healthier metabolic status of the subjects in the study of Yoshino *et al.*[6,7]

In conclusion, resveratrol supplementation for 30 days induced a shift toward an increased proportion of small adipocytes. This phenotype was accompanied by a gene expression profile indicative of increased adipogenesis, an alternative pathway of lipid breakdown by autophagy and an increased immune and inflammatory response. Further research is necessary to investigate whether resveratrol improves adipose tissue function.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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